Ecotoxicology and Environmental Safety 74 (2011) 1363–1369

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Exposure of juvenile green frogs (*Lithobates clamitans*) in littoral enclosures to a glyphosate-based herbicide

Christopher B. Edge [a,*], Megan K. Gahl [a], Bruce D. Pauli [b], Dean G. Thompson [c], Jeff E. Houlahan [a]

[a] Canadian Rivers Institute, Department of Biology, University of New Brunswick, Saint John, New Brunswick, Canada E2L 4L5
[b] National Wildlife Research Center, Environment Canada, Carleton University, Ottawa, Ontario, Canada K1A 0H3
[c] Great Lakes Forestry Center, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada P6A 2E5

ARTICLE INFO

Article history:
Received 30 November 2010
Received in revised form
5 March 2011
Accepted 18 April 2011
Available online 4 May 2011

Keywords:
Rana clamitans
Lithobates clamitans
Glyphosate
Roundup
Herbicide
Batrachochytrium dendrobatidis

ABSTRACT

The majority of studies on the toxicity of glyphosate-based herbicides to amphibians have focused on larval life stages exposed in aqueous media. However, adult and juvenile amphibians may also be exposed directly or indirectly to herbicides. The potential for such exposures is of particular interest in the littoral zone surrounding wetlands as this is preferred habitat for many amphibian species. Moreover, it may be argued that potential herbicide effects on juvenile or adult amphibians could have comparatively greater influence on overall recruitment, reproductive potential and thus stability of local populations than effects on larvae. In this experiment, juvenile green frogs (*Lithobates clamitans*) were exposed to two concentrations (2.16 and 4.27 kg a.e./ha) of a glyphosate-based herbicide formulation (VisionMax®), which were based on typical application scenarios in Canadian forestry. The experimental design employed frogs inhabiting *in situ* enclosures established at the edge of small naturalized wetlands that were split in half using an impermeable plastic barrier. When analyzed using nominal target application rates, exposure to the glyphosate-based herbicide had no significant effect on survival, body condition, liver somatic index or the observed rate of *Batrachochytrium dendrobatidis* infection. However, there were marginal trends in both ANOVA analysis and post-hoc regressions regarding *B. dendrobatidis* infection rates and liver somatic index in relation to measured exposure estimates. Results from this study highlight the importance of field research and the need to include multiple endpoints when examining potential effects of a contaminant on non-target organisms.

1. Introduction

Amphibian populations are declining around the world (Houlahan et al., 2000; Stuart et al., 2004) including some in environments that are considered relatively pristine. An array of possible causal factors, including habitat destruction, climate change, disease, chemical contaminants, invasive species, and interactions among one or more of these factors have been postulated to explain these declines. Among the multitude of potential causal factors, disease and pesticides have garnered much of the recent scientific attention (Kilpatrick et al., 2010; Davidson, 2004).

Studies on the effect of pesticides on amphibians have focused on some of the more widely used compounds such as the herbicides glyphosate and atrazine. Glyphosate-based herbicides are currently the dominant herbicides in both agriculture and silviculture (Thompson et al., 2010; Duke and Powles, 2008; Woodburn, 2000). Glyphosate, the active ingredient, is considered to be relatively non-toxic to non-target vertebrate and invertebrate species (Giesy et al., 2000), largely because the active ingredient (glyphosate isopropylamine salt) inhibits the activity of enolpyruvylshikimate phosphate synthase, an enzyme found only in plants and a few microbial species (Rubin et al., 1982). However, multiple laboratory studies have shown that formulated end-use products containing glyphosate as the active ingredient are moderately to highly toxic to amphibian larvae at concentrations between 1 and 4 mg acid equivalents (a.e.)/L (Relyea and Jones, 2009; Cauble and Wagner, 2005; Relyea, 2005a; Edginton et al., 2004; Howe et al., 2004; Mann and Bidwell, 1999). The observed toxicity of formulated products is generally attributed to the inclusion of surfactants and particularly the polyethoxylated tallowamine surfactant (POEA) (Edginton et al., 2004; Howe et al., 2004; Solomon and Thompson, 2003; Giesy et al., 2000, Mann and Bidwell, 1999) as this surfactant is cytotoxic and disrupts the membrane of sensitive respiratory surfaces (Partearroya et al., 1991).

However, field experiments (Thompson et al., 2006; Wojtaszek et al., 2004) and some mesocosm experiments (Bernal et al., 2009a)

* Corresponding author.
E-mail addresses: christopher.edge@unb.ca (C.B. Edge), gahlm@unb.ca (M.K. Gahl), bruce.pauli@ec.gc.ca (B.D. Pauli), dean.thompson@nrcan-rncan.gc.ca (D.G. Thompson), jeffhoul@unbsj.ca (J.E. Houlahan).

0147-6513/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2011.04.020

conducted using formulated glyphosate products at lower concentrations (e.g. between 0.21 and 3 mg a.e./L) show no statistically significant acute toxicity or sublethal effects on growth, avoidance behavior or other commonly studied endpoints. Despite all of this recent research activity, there remains a general paucity of manipulative experiments on amphibians under more natural environmental conditions. Further, only a few studies investigating the effects of glyphosate-based herbicides on post-metamorphic amphibians (Bernal et al., 2009b; Dinehart et al., 2009; Relyea, 2005b) have been published. Terrestrial and semi-aquatic life stages of amphibians are important in nutrient and energy cycling (Verburg et al., 2007; Beard et al., 2002; Travis, 1984), may exert top–down effects on invertebrate prey species (Greenless et al., 2006; Beard et al., 2003), and serve as a significant food source for other vertebrates and invertebrates. It is these animals which reproduce and thus contribute to the persistence of local populations. Therefore, understanding the potential impacts of herbicide applications on juvenile and adult life stages of amphibians, either alone or in combination with other natural or anthropogenic stressors, is important for conservation of populations and the structure and function of ecosystems.

Post-metamorphic amphibians that inhabit the littoral region of wetland ecosystems may be exposed to herbicides through direct overspray or spray drift from ground-based and aerial applications. Direct overspray of post-metamorphic animals of three species of North American amphibians with a glyphosate-based formulation (Roundup Weed and Grass Killer) at an application rate of 10.83 kg a.e./ha, (Relyea, personal communication; Honegger, personal communication) resulted in near complete mortality in one laboratory experiment (Relyea, 2005b). In addition, increased mortality was observed in another study on two species of North American toads using another formulation (Roundup Weed and Grass Killer Ready-To-Use Plus) applied at 9.84 kg a.e./ha (Dinehart et al., 2009). However, one other glyphosate-based herbicide, Roundup WeatherMax applied at a lower application rate (1.73 kg a.e./ha), did not cause increased mortality in the two species of toads (Dinehart et al., 2009). In a study of several South American amphibian species exposed to a different glyphosate-based formulation (Glyphos Cosmo-Flux mixture), mortality was observed when the herbicide was applied at application rates of 1.85 and 3.69 kg a.e./ha (Bernal et al., 2009b). One important factor in the Bernal et al. (2009b) study was the inclusion of soil and leaf litter as a substrate, as the toxicity of glyphosate-based herbicides to post-metamorphic amphibians may be mitigated by the presence of soil (Dinehart et al., 2009). The majority of glyphosate formulations tested to date on post-metamorphic amphibians are products designed for household use and only one formulation (Roundup WeatherMax) is routinely used in agriculture (in North America). In large-scale applications in agriculture or forestry, herbicides are typically applied by aircraft or large mechanical spray delivery units. With these application methods there is greater potential for direct overspray of adult and juvenile amphibian species in the littoral region of ponds than with handheld or backpack sprayers used in household applications. To our knowledge, there have been no field studies conducted on the effects of glyphosate formulations commonly used in agriculture or forest management on post-metamorphic amphibians, and none with the formulated product VisionMax®, which is commonly used in Canadian forest management.

A variety of effects may result when amphibians are exposed to chemicals, including acute and chronic mortality or sublethal effects such as reduced energy stores or increased activity of detoxifying organs (e.g., liver). Beyond direct effects on body condition and survival, chemical stressors could also impact amphibian immune systems, making them more susceptible to disease (Carey et al., 1999). A disease that has received attention because it has been linked to amphibian declines around the world is chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) (Kilpatrick et al., 2010; Daszak et al., 1999). Chytridiomycosis affects the skin of post-metamorphic amphibians (Longcore et al., 2007), and antimicrobial skin peptides may be a key factor in protecting post-metamorphic amphibians from chytrid infection (Rollins-Smith et al., 2002). It is plausible that a chemical sprayed on amphibian skin may affect these skin peptides and hinder the immunological function of peptides against chytrid infection. Furthermore, some pesticides have been shown to cause immune suppression in amphibians (Christin et al., 2004; Taylor et al., 1999). However, no available study has investigated the potential effect of widely used glyphosate-based herbicides on amphibian disease incidence.

In this study, we examined the effects of a glyphosate-based herbicide (VisionMax) on post-metamorphic amphibians in the setting of a field experiment. We used *in situ* littoral enclosures within a split-wetland experiment to investigate the effects of the herbicide at two operationally relevant treatment concentrations on juvenile green frogs (*Lithobates clamitans*; formerly *Rana clamitans*). We considered four endpoints: survival, changes in body condition (mass related to length), liver somatic index, and the observed incidence of disease (infection by the fungal pathogen *Batrachochytrium dendrobatidis*) in animals exposed to a glyphosate-based herbicide relative to untreated, control animals.

2. Materials and methods

2.1. Experimental design

The study was carried out at the Long-Term Experimental Wetlands Area (LEWA) on Canadian Forces Base Gagetown in New Brunswick, Canada. As part of a larger ongoing experiment, 10 permanent wetlands were divided in half using an impermeable high density polyethylene (HDPE) barrier. On each side of the divided wetland, one enclosure ($0.8\ m \times 2\ m$, $1.6\ m^2$) was constructed from aluminum flashing and 3.2 mm HDPE mesh. The two long sides and one short side were constructed of aluminum and the remaining short side was constructed of mesh. Each enclosure had a removable mesh cover held in place with Velcro. Enclosures were situated so that half of the enclosure was terrestrial and the other half aquatic, with the mesh side partially submerged in the water to allow for water circulation (Fig. 1). In each enclosure we randomly assigned and placed 5 first-year green frogs in each enclosure. Each animal was weighed ($\pm$0.1 g) using a 10 or 100 g spring scale (Pesola, Rebmattli, Baar, Switzerland) and snout vent length (SVL) was measured using a ruler. One side of each wetland was treated with the herbicide VisionMax® (540 g a.e./L, Monsanto, Winnipeg, MB, CAN) and the other was left as an untreated control (see below). This "split-wetland" design helps control for intrinsic differences that arise when making comparisons between wetlands.

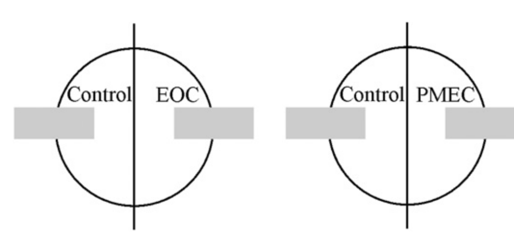

Fig. 1. Line diagram of experimental setup. Wetlands were split in half using an impermeable plastic barrier and one enclosure (shaded area) was constructed on each side of the barrier. One side of each wetland was treated with a glyphosate-based herbicide, while the other side was left as an untreated control. Two nominal application rates were used: (1) Environmentally Observed Concentration (EOC), 2.16 kg a.e./ha and (2) Predicted Maximum Environmental Concentration (PMEC), 4.27 kg a.e./ha. Data from four replicates of each treatment were analyzed (see results).